Physiological Monitoring in Diving Mammals

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LONG-TERM GOALS

The objective with this study is to develop and calibrate an invasive data logger to measure muscle O_2 saturation in large, freely diving whales. We intend to use this data logger to measure muscle O_2 saturation and determine how blood flow to muscle is altered during diving. These data will be important to determine if muscle blood flow is reduced during diving, and important to estimate how the dive response affects muscle N_2 levels and the risk of decompression sickness (DCS).

OBJECTIVES

This project is separated into two aims:

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Aim 1: Develop a new generation of tags/data logger for marine mammals that will contain a sensor to be implanted into the muscle. The logger will collect physiological data from muscle tissue in freely diving marine mammals. The sensor will be tested and calibrated in terrestrial mammals at Massachusetts General Hospital, Boston.

Aim 2: The data logger will be tested in freely diving marine mammals in the field, and muscle O_2 saturation data will be collected.

APPROACH

This project is separated into two aims: Aim 1a) Development of a new generation of tag/data logger for marine mammals that will contain a sensor to be implanted into the muscle. The logger will collect physiological data from muscle tissue in freely diving marine mammals. The sensor will be tested and calibrated in terrestrial mammals at Massachussetts General Hospital, Boston; Improve the ; Aim 2) The data logger will be tested in freely diving marine mammals in the field, and muscle O_2 saturation data will be collected.

Aim 1: A near infrared spectrophotometer connected to a data logging device will be developed and used to measure myoglobin/hemoglobin O_2 saturation in freely deep diving whales (e.g. beaked whales, sperm whales, Fig. 1). The unit will be developed based upon the succesful construction of an oximeter used in Weddel seals [1].

A delivery device will be fabricated to allow implantation of the optical probe into the muscle. The flexible cable will allow the muscle to move freely, resulting in minimal discomfort. Initial experiments on terrestrial mammals and stranded or by-caught (post-mortem) marine mammals will assess the impact of the implantation to minimize the potential for inflammation and hematoma [2].

Aim 2: The data logger will be tested on a variety of diving marine mammals over 2 field seasons. We aim to perform trans-location experiments in Northern elephant seals (*Mirounga angustirostris*) with collaborators at University of California Santa Cruz (UCSC). This allows us to perform controlled field experiments and to determine if the data logger is able to collect the physiological data and to assure minimal impact to the animal.

We will implant the oximeter into the muscle of freely, deep diving whales (beaked and/or sperm whales) during 2 field seasons.

WORK COMPLETED

Aim 1:
Figure 1 shows the overall concept of tag attachment (courtesy UFI).

LED/photosensor development: In the fall 2011 we fielded test the LED and photosensors (Fig. 2) before putting them into the tag housing (Fig. 1).

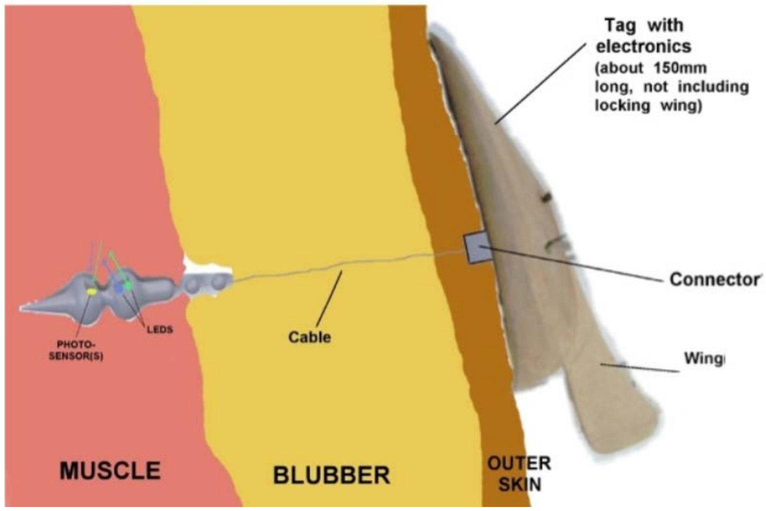

Figure 1. Muscle implanted ballistic containing the LED and photosensor, which will be connected to the external data logger

The photos below show the setup to test and calibrate the electronics at pressure and for different O_2 concentrations (0% and 100% O_2). The electronics is inserted into a pressure vessel that is hydraulically compressed. The output from the sensors on the board; acceleration, bearing, and pressure, are output to a computer. We have shown that all components are able to withstand a pressure of 3000 m, but there are some non-linearities in the pressure sensor at pressures > 2000m. We are currently working to resolve that.

In Fig. 2 one of the ballistic ends that we are testing is shown. Also shown is the test rig to oxygenate and deoxygenate blood and muscle to test the system. The LED and photo sensor is attached to the ballistic end and these are inserted into the muscle. The muscle is flushed with O_2 or N_2 to either remove or add O_2. One photo shows the tissue volume that is illuminated by the LED. The photo shows that we are able to view a considerbale volume of muscle, thus allowing the O_2 saturation to be measured accurately. The voltage was read by a voltmeter but is currently being integrated into the data logger electronics (Fig. 2). We have shown that the system responds when we flush the muscle with O_2 or N_2. Calibration in live sheep will be performed at MGH by the Zapol group in the fall 2012.

We aim to remove the ballistic end from the muscle at the end of the experiment and we have therefore tested several ballistic ends. To determine if the hydrodynamic drag of the data logger housing will be able to pull the sensor end out of the muscle, we performed drag tests of the logger housing. The housing was pulled after a boat at different speeds and the force was measured.

THE BOAT TEST OF TAG COMING OUT IN THE WATER

Boat speed (mph)	Scale reading (kg)
5	0.8
6	1.6
7	2.0
8	3.0
9	4.0

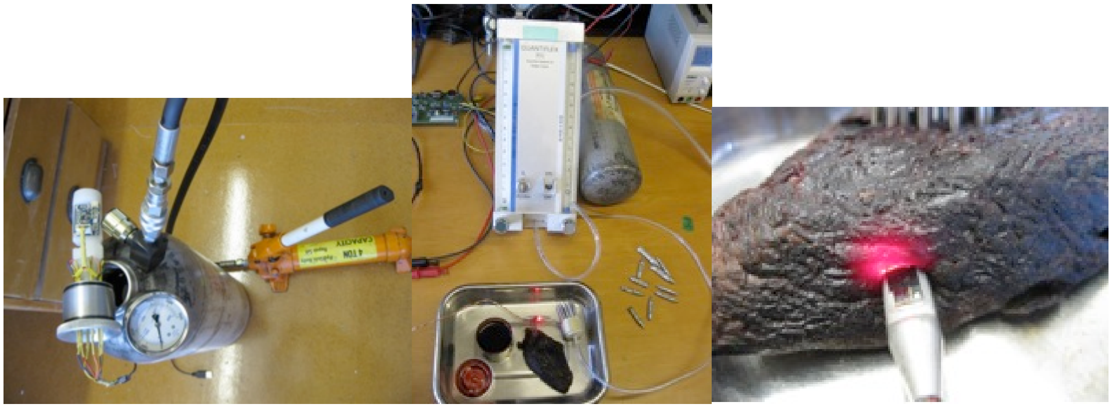

Figure 2. Setup to pressure test LED and photosensors and to test absorbance changes in muscle tissue when oxy-deoxygenated

In January 2012, one of our collaborators, Mr. Trevor Austin at Paxarms, worked with Department of Conservation in New Zealand during a mass-strading of pilot whales. During this stranding event we had the opportunity to test that we can implant the ballistic end into the muscle, using a rifle, without damaging the LED and photosensor. The experiment also allowed us to test that we can place the wire that connects the ballistic end and the data logger in the interface between the blubber ad muscle. We managed to do this on two animals and received data as the O_2 was depleted in the muscle of the recently dead animals (Fig. 3).

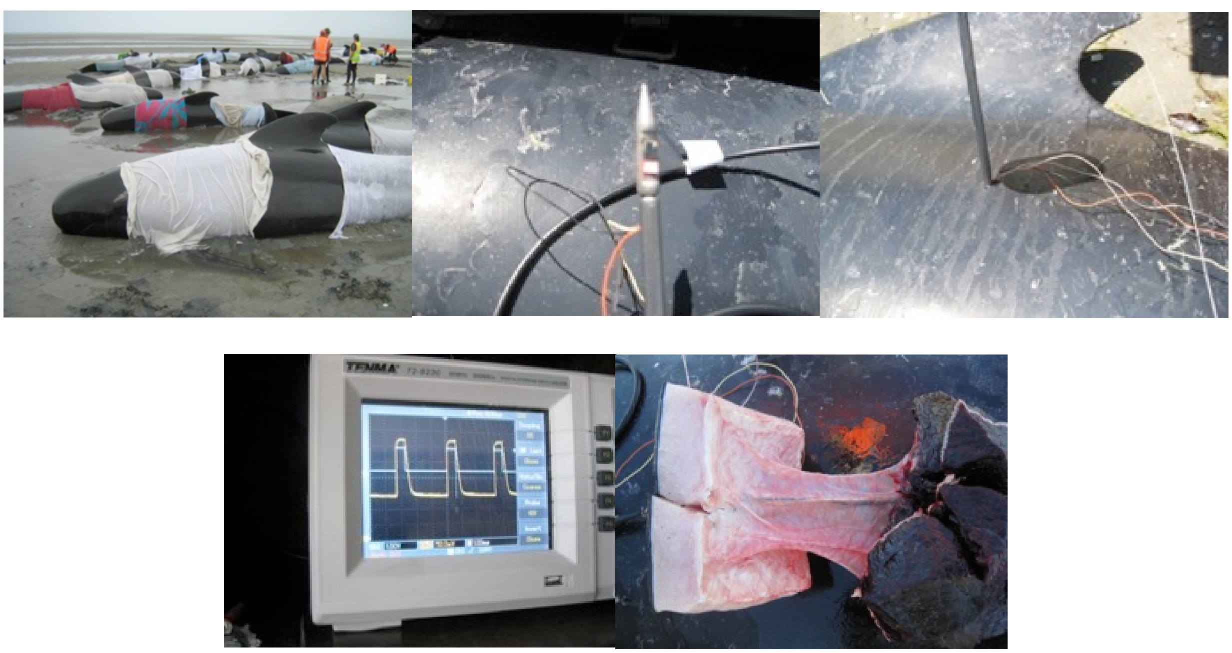

Figure 3. Field testing of oximeter sensor and implant device in stranded/deceased pilot whales.

Additional field testing will continue in the next year, when we will attach the calibrated data logger and implant the ballistic head in recently deceased stranded whales.

Data logger: We are working with the electronics department at the University of Canterbury in Christchurch New Zealand to develop the data logger and sensors. A student has been working on the board design (Fig. 4) and the finished logger will be ready in the fall 2012. The data logger and oximeter ends will be shipped to the US during the fall 2012 where it will be calibrated and tested before field testing on elephant seals in the spring 2013.

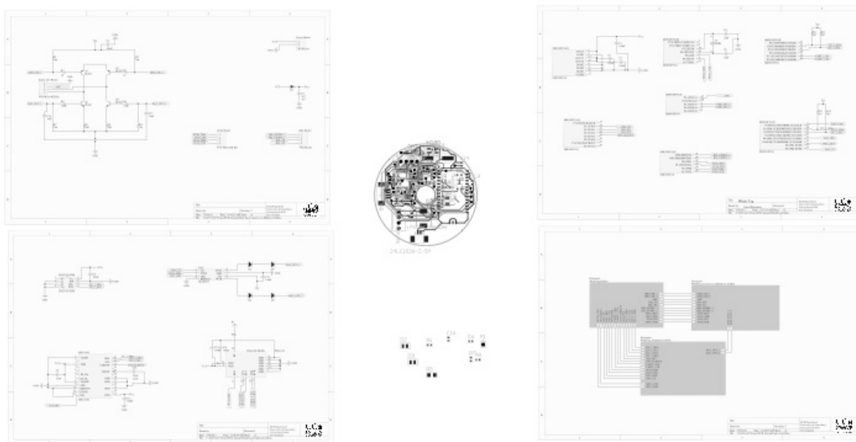

Figure 4. Schematics of electronics board for data logger.

Implantation and delivery system: Initial measurements indicated that there is a lot of lateral movement between the blubber and muscle layers in whales. We therefore aim to attach the LED and photosensor via a ballistic device, which will be connected to the external data logger (Fig. 1).

The data logger/oximeter sensor package is being delivered using a modified rifle (Fig. 5). The system was tested on stranded post-mortem pilot whales. These tests verified that the forces during impact did not damage the sensor. In addition, the wire was placed in the blubber/muscle interface (Fig. 6) allowing lateral movement of the two interfaces without damaging the tissues or moving the sensor.

[Figure 5. Prototype of rifle, Paxarms Remote Delivery System (PRDS) that will be used to implant the oximeter probe into the muscle. The PRDS consist of a rifle with a scope and a guideline.]

[Figure 6. Dissection of deceased pilot whale following implantation of ballistic sensor end and wire.]

Additional testing and development: For the oximeter used in previous studies [1, 3] a basic assumption was that the tissue scattering is negligible. This assumption may not be true for biological tissue and we therefore are testing new technology which we hope will collect additional information and allow us to quantify absorption and scattering without assumptions and additional measurements or calibration.

Aim 2: We expect to have the first prototype of the probe and logger ready for testing on post-mortem stranded whales in the January 2013, and we are planning translocation experiments in Santa Cruz in April 2013.

We consider the Northern elephant seal to be a suitable species to perform the first tests. This species perform long and deep dives, similar to sperm and beaked whales. It is also possible to perform controlled translocation experiments in this species where the instruments can be retrieved within a few days of release. For the field experiments on elephant seals, we are planning experiments in collaboration with researchers at UCSC in Santa Cruz.

For tag deployment on whales, we have developed collaboration with Norwegian colleagues at the Norwegian Defense Research Establishment in Horten and the Polar Institute in Tromsø, Norway. If the translocation experiments on elephant seals and the field testing on recently deceased whales in New Zealand are successful, we will plan tagging experiments on live whales.

RESULTS

Aim 1: All the components of the data logger (oximeter probe and data logger housing and electronics) and delivery system (custom-built rifle) are currently in the development stage. The electronics and oximeter sensor will be tested and calibrated in the fall 2012. We have shown that we are able to detect differences in blood oxygenation using LEDs and reflectance sensors and the selected sensors are able

to cover a muscle volume large enough to allow accurate detection of the O_2 saturation. We have shown that we can deliver these into the muscle of a pilot whale.

Aim 2: We are planning translocation experiments and additional field expeirments on stranded animals in year 2 and all the animal care protocols and permits have been approved.

IMPACT/APPLICATIONS

This work is intended to enhance our understanding of how the dive response alters muscle blood flow and metabolism in large, freely diving whales. The results will provide information that will enable more realistic predictions of how the dive response varies during breath-hold diving at different activities. The study will also provide a new generation of data loggers that are able to collect physiological data in large whales with minimal impact.

Results from the completed study will help to improve our understanding about the physiology of marine mammals and improve modeling efforts that are aimed at estimating inert gas levels in breath-hold divers. The results can be used to determine how changes in dive behavior, from playback studies that measures avoidance patterns in deep diving whales, affect blood and tissue P_{N_2} levels. Thus, our results will enhance the fundamental understanding, interpretation and avoidance of the effect of anthropogenic sound, and enable knowledgeable decisions about sonar deployment, related training exercises and responses to NGO concerns. This should be of value to the US Navy Marine Mammal Program.

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